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FULL RESEARCH PAPER

Vegetative compatibility groups in *Verticillium dahliae* isolates from olive in western Turkey

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Abstract Verticillium wilt, caused by *Verticillium dahliae*, is the most serious disease in olive cultivation areas in western Turkey. Two hundred and eight isolates of *V. dahliae* from olive (*Olea europea* var. *sativa*) trees were taken for vegetative compatibility analysis using nitrate non-utilizing (*nit*) mutants. One isolate did not produce a *nit* mutant. *Nit* mutants of 207 isolates were tested against tester strains of internationally known vegetative compatibility groups (VCGs) 1A, 2A, 2B, 3, 4A and 4B, and also paired in many combinations among themselves. One hundred and eighty nine of the isolates (90.9%) were strongly compatible with T9, the tester strain of VCG1A, and thus were assigned to VCG1A. Eight isolates were assigned to VCG2A and four isolates to VCG4B. One isolate was heterokaryon self-incompatible (HSI) and five isolates could not be grouped to any of the VCGs tested. Pathogenicity assays were conducted on a susceptible olive cultivar (*O. europea* cv. Manzanilla) and a susceptible local cotton cultivar (*Gossypium hirsutum* cv. Çukurova 1518). Both cotton and olive inoculated with all VCG1A isolates showed defoliating symptoms in greenhouse tests. This is the first report on VCGs in *V. dahliae* from olive trees in Turkey which demonstrates that VCG1A of the cotton-defoliating type is the most commonly detected form from olive plants in the western part of Turkey.

Keywords Verticillium wilt · Olive · Turkey · Vegetative compatibility groups · Pathogenicity · Cotton defoliating · Olive defoliating

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Introduction

Olive (*Olea europea* var. *sativa*) trees are grown across the Mediterranean countries. In Turkey, there are 107 million olive trees that occupy over 649,000 ha (Anonymous 2005). This corresponds to 15.8% of the whole arboricultural cultivation in the country (Anonymous 2005). Olive trees are grown extensively and intensively mainly in western Turkey.

Verticillium wilt caused by the fungus *Verticillium dahliae* is one of the most important diseases

occurring in olive cultivation all over the world. The disease on olive was first reported in Turkey in 1972 (Saydam and Copcu 1972). Economic damage caused by the wilt has increased during the past decade since new orchards are established in fields previously cropped with susceptible hosts of the pathogen (Blanco-López et al. 1984). Recently, *V. dahliae* has infected a high number of olive fields in the western Anatolian region (Benlioğlu et al. 2001; Yolageldi et al. 2001). In Manisa province, there are approximately 12 million olive trees and 5% of them are infected with Verticillium wilt. About half of the trees are concentrated in the Akhisar and Gölmarmara districts and 8–10% of these trees are infected (L. Erten, unpublished). In the Aydın and Muğla provinces, it is estimated that there are approximately 13 to 20 million olive trees of which 5 to 10% are infected with Verticillium wilt (Benlioğlu et al. 2001; L. Erten, unpublished). The disease is common in these areas, substantially reduces the production of olive orchards and may cause tree deaths. However, in the Balıkesir province where almost all of 10 million olive trees are located in and around Edremit Bay the occurrence of the disease is very rare (L. Erten, unpublished).

Since *V. dahliae* is a strictly asexually reproducing fungus (Pegg and Brady 2002), vegetative compatibility is a prerequisite to genetic exchange among different strains of the pathogen. Therefore, *V. dahliae* isolates in different vegetative compatibility groups (VCGs) are thought to be genetically isolated populations which all have the potential to share a common gene pool which may vary in many characteristics (Katan 2000).

VCG1, VCG2, VCG3 and VCG4 were identified among *V. dahliae* isolates from diverse geographic and plant sources worldwide by using nitrate-non-utilizing (*nit*) mutants (Bell 1994; Chandelier et al. 2003; Chen 1994; Daayf et al. 1995; Joaquim and Rowe 1991; Korolev et al. 2000, 2001a; Strausbaugh 1993; Zhengjun et al. 1998). Each of VCG1, VCG2 and VCG4 was further divided into two subgroups designated as A and B (Bell 1994; Joaquim and Rowe 1991; Strausbaugh 1993). Recently, VCG6 has been identified among *V. dahliae* isolates infecting pepper in California (Bhat et al. 2003).

Verticillium dahliae infecting olive can be classified as defoliating (D) and non-defoliating (ND) pathotypes according to their ability to defoliate the plant (López-Escudero et al. 2004). All the D strains (from America, China and Spain) tested to date belong to VCG1 subgroup A (Bell 1994; Korolev et al. 2001a). This differential virulence is exhibited in cotton (*Gossypium hirsutum*), with isolates from cotton and olive with cross-virulence (Schnathorst and Sibbett 1971).

Natural infections by the D pathotype were found in olive orchards in Spain (López-Escudero and Blanco-López 2001; Mercado-Blanco et al. 2003), but not in other olive-growing countries of the Mediterranean basin. While infections by the D pathotype can be lethal to the plant, olive plants infected with the ND pathotype can recover (Jiménez-Díaz et al. 1998; López-Escudero and Blanco-López 2005; Mercado-Blanco et al. 2001). Several efforts have focused on a resistance screening programme in olive cultivars. López-Escudero et al. (2004) found 23 olive cultivars to be more susceptible or extremely susceptible to the D pathotype than the ND pathotype. Martos-Moreno et al. (2006) found that 26 of the 33 major olive cultivars were susceptible or extremely susceptible, while seven other olive cultivars were found to be moderately or completely resistant to the D pathotype of *V. dahliae*. Therefore, the proper characterization of *V. dahliae* pathotypes infecting olive is of great importance in controlling the disease.

In the present work, we aimed to determine (1) VCG diversity in *V. dahliae* isolates from olive trees grown in western Turkey, (2) the relatedness of Turkish VCGs to internationally recognized VCGs and (3) the aggressiveness of isolates from different VCGs on olive and cotton plants.

Materials and methods

Collection of isolates

The isolates originating from 208 sites in 45 districts of seven provinces in the western part of Turkey were collected from olive trees with wilt symptoms between 2003 and 2005 (Table 1). Pieces of vascular tissue from branch segments (2 cm long) were dipped in 70% ethanol, surface-sterilized with 1% NaOCl for 1 min, rinsed in sterile distilled water (SDW) and dried on sterile filter paper. Each segment was then placed on potato dextrose agar (PDA Merck, Damstradt, Germany) amended with 100 mg l^{-1} of streptomycin

Table 1 Vegetative compatibility groups (VCGs) of isolates of *V. dahliae* from olives in western Turkey

Geographical origin		VCG	Isolate codes
Province	District		
Aydın	Merkez	1A	OVd39, 228
	Bozdoğan	1A	OVd89, 98, 124, 126, 128
	Çine	1A	OVd205, 113
	Germencik	1A	OVd45, 36
	Karacasu	1A	OVd44, 85, 100, 101, 107, 129, 230
	Koçarlı	1A	OVd48, 95, 122
	Köşk	1A	OVd42, 97, 104, 120, 121, 125, 127
	Kuşadası	1A	OVd84, 88, 96, 102, 106, 210, 118, 116
		2A	OVd208
		NC	OVd38
	Nazilli	1A	OVd111, 94, 46
	Söke	1A	OVd41, 223
	Sultanhisar	1A	OVd90, 231
	Kuyucak	1A	OVd86, 87, 91, 93, 99, 103, 105, 109, 112, 114, 115, 123, 209, 92, 110, 117
	Yenipazar	1A	OVd35, 40, 108, 119
Balıkesir	Ayvalık	1A	OVd227
Bursa	Gemlik	2A	OVd232
	Karacabey	2A	OVd206, 202
	Mudanya	2A	OVd204, 234
Denizli	Merkez	1A	OVd199, 224
İzmir	Aliağa	4B	OVd31, 57, 60
	Urla	1A	OVd219
	Bayındır	1A	OVd61, 77, 78, 203, 58
	Bornova	1A	OVd62, 32, 67
	Buca	1A	OVd74
	Dikili	NC	OVd221
	Foça	1A	OVd69
		4B	OVd56
	Gaziemir	1A	OVd80
	Kemalpaşa	1A	OVd21, 216, 64, 68, 27, 30, 75, 73, 217
		HSI	OVd55
	Kiraz	1A	OVd82
	Ödemiş	1A	OVd214, 212, 213, 215, 220
	Selçuk	1A	OVd23
	Tire	1A	OVd83, 79, 81, 34
	Torbalı	1A	OVd76, 218, 28, 63
	Konak	1A	OVd26
Manisa	Akhisar	1A	OVd49, 6, 47, 54, 131, 160, 157
		2A	OVd151
	Gölmarmara	1A	OVd135, 139, 144, 145, 147, 155, 166, 168, 170, 173, 174, 175, 178, 52, 133, 161, 162, 141, 159, 136, 158, 167, 142, 146, 148, 149, 150, 152, 172, 180, 229, 130, 132, 140, 164, 171, 177, 163, 179, 134, 137, 138, 143, 154, 169, 176, 233
		NC	OVd53
	Kırkağaç	1A	OVd22, 37
	Saruhanlı	1A	OVd50, 165
	Selendi	1A	OVd51, 156
	Soma	1A	OVd222
	Merkez	1A	OVd153
	Turgutlu	NM	OVd71
Muğla	Bodrum	1A	OVd187, 189
	Milas	1A	OVd8, 182, 183, 186, 188, 190, 181, 13, 12, 11, 10, 14, 15, 184, 226, 207, 225, 185, 15, 17, 7, 9
		NC	OVd18, 16
	Yatağan	2A	OVd211

HSI Heterokaryon self-incompatible

NC Non-characterized. *Nit* mutants obtained from these isolates did not produce a strong heterokaryon with the local and international tester isolates of four VCGs.
NM did not produce a *nit* mutant

sulphate. Plates were incubated at 24°C for 5–7 days. *Verticillium dahliae* was identified on the basis of its morphological features according to the description of Smith (1965). Sixty-five isolates were obtained from 13 districts in the Aydın province, one from Ayvalık district in the Balıkesir province, five from three districts in the Bursa province, two from the Merkez district in the Denizli Province, 43 from 16 districts in the İzmir province, 65 from 8 districts in the Manisa province and 27 from three districts in the Muðla province of western Turkey (Table 1). The major olive cultivars grown in these provinces were Manzanilla, Domat, Ayvalık, Gemlik, Memecik and Uslu. Monoconidial isolation of the fungus was performed using the method described previously (Bell 1992). A total of 208 single-spored isolates of *V. dahliae* each of which was obtained from different sites was stored on PDA at 4°C and used for VCG characterization.

Generation and characterization of nitrate-non-utilizing (*nit*) mutants

Water agar-chlorate medium (WAC) was used to select *nit* mutants. This medium was based on water agar (2%) amended with 0.02% glucose and 2.5–5% potassium chlorate (Korolev and Katan 1997). Mycelial plugs (about 1 mm^2) cut from the edge of the monoconidial cultures were placed on WAC in the centre in 9 cm Petri dishes, and incubated at 25°C with a 12-h photoperiod of fluorescent and near-UV light at 36 μEm^{-2} s^{-1} for up to 4 weeks. Chlorate-resistant sectors, which appear as thin fast- growing mycelial sectors or as fan-like sectors at the colony perimeter after 10–28 days, were transferred to Czapek-Dox agar (CDA; Merck, Damstradt, Germany) Petri dishes (5-cm diameter) and allowed to grow for 5 days. Only sectors that grew on CDA as colonies with a thin, expansive mycelium were considered *nit* mutants.

CDA amended with sodium nitrite (0.5 g l^{-1}) or hypoxanthine (0.2 g l^{-1}) was used for partial phenotyping of the *nit* mutants (Correll et al. 1987). Mutants that grew profusely (similar to wild-type) on CDA with nitrite or hypoxanthine were classified as *nit*1. Mutants that grew sparsely on CDA with hypoxanthine were classified as NitM. Mutants that grew profusely on hypoxanthine and sparsely on nitrite were classified as *nit*3. These partially phenotyped *nit* mutants were labelled and stored for future use.

Complementation and vegetative compatibility

This part of the work was carried out in two stages. In the first set of experiments, *nit* mutants of all isolates were paired with complementary *nit* mutants of the international and Turkish tester isolates of previously described VCGs (Table 2). The *nit*1 and NitM mutants from each isolate were also paired with each other to test for heterokaryon self-compatibility. Complementation was tested on CDA. Generally, each 5-cm diameter Petri dish was inoculated with three mutants, 1 cm apart in a triangular pattern, and incubated for 28 days at 24°C in the dark. Pairings were scored for prototrophic growth 7–28 days after inoculation. Complementation was indicated by the formation of a dense, aerial growth or black microsclerotia where mycelia of an unknown and a tester strain had met and formed a prototrophic heterokaryon (Correll et al. 1987). In this case, the unknown mutant was considered vegetatively compatible with the tester strain and was assigned to the VCG of the tester strain. Each pairing was performed at least twice.

In the second stage, 10 isolates belonging to local VCGs and recovered from olive trees in different

Table 2 Summary information about previously described international and Turkish tester isolates of *V. dahliae* used in this study

Isolate	Origin	Mutant phenotype	VCG	Host plant	Reference
T9	USA	NitM	1A	Cotton	Joaquim and Rowe (1991)
cot228	Israel	*nit1*	1	Cotton	Korolev et al. (2001b)
ep8	Israel	NitM	2A	Eggplant	Korolev et al. (2000)
ep52	Israel	*nit*1	2A	Eggplant	Korolev et al. (2000)
Cka1	Turkey	*nit*1 and NitM	2A	Cotton	Dervis and Bicici (2005)
Ch02	Turkey	*nit*1 and NitM	2A	Cotton	Dervis and Bicici (2005)
Cy1	Turkey	*nit*1 and NitM	2A	Cotton	Dervis and Bicici (2005)
cot256	Israel	*nit*1	2B	Cotton	Korolev et al. (2000)
cot11	Israel	NitM	2B	Cotton	Korolev et al. (2000)
Ch01	Turkey	*nit*1 and NitM	2B	Cotton	Dervis and Bicici (2005)
Ch03	Turkey	*nit*1 and NitM	2B	Cotton	Dervis and Bicici (2005)
70-21	USA	NitM	3	Pepper	Joaquim and Rowe (1991)
171	USA	*nit*1	4A	Potato	Joaquim and Rowe (1991)
131M	USA	NitM	4A	Potato	Joaquim and Rowe (1991)
Pt15M	Israel	NitM	4B	Potato	Korolev et al. (2000)
Pt9G	Israel	*nit*1	4B	Potato	Korolev et al. (2000)
Cy4	Turkey	*nit*1 and NitM	4B	Cotton	Dervis and Bicici (2005)
Cko1	Turkey	*nit*1 and NitM	4B	Cotton	Dervis and Bicici (2005)

provinces of Turkey were used as the set of local tester strains (Table 3). An isolate obtained from a severely diseased cotton plant in Manisa which complemented strongly with T9 was also included in the local tester set. These isolates yielded strong heterokaryons with the corresponding international testers shown by both NitM and *nit*1 mutants. *Nit* mutants of other local isolates were paired with *nit* mutants of the local tester isolates to observe the homogeneity among the local isolates belonging to the same VCG.

Pathogenicity tests

Two independent experiments (I and II) were carried out in Hatay and İzmir using VCG-characterized isolates from olive. In experiment I, isolates were tested on cotton to determine pathogenicity of different VCGs. The experiment included 17 randomly selected isolates within different VCGs from olive, 12 of which belonged to VCG1A (OVd7, OVd23, OVd34, OVd54, OVd81, OVd82, OVd103, OVd110, OVd146, OVd154, OVd173 and OVd223) three to VCG4B (OVd56, OVd57 and OVd60), one to VCG2A (OVd151), and one was HSI (OVd55).

Disinfested cottonseeds (*G. hirsutum* cv. Çukurova 1518) were sown in trays filled with non-sterilized sandy soil. Plants were grown in a greenhouse at 22–26°C, with 14-h photoperiod of fluorescent light of 270 $\mu Em^{-2}s^{-1}$. Relative humidity in the greenhouse ranged from 50 to 90% during the light period and from 60 to 100% during the dark period. Seedlings were uprooted from the soil 2–4 days after emergence (7–14 days after sowing). Their roots were washed free of soil, trimmed and dipped in the inoculum suspension (10^6 conidia ml^{-1}) for 3 min. Non-inoculated control seedlings were dipped in SDW. Seedlings were then transplanted (five per pot) into 12-cm diameter plastic pots filled with non-sterilized potting mixture (clay loam/peat; 2:1, *v*/*v*), and maintained in the greenhouse in a completely randomized design. Plants were watered as needed and fertilized every 2 weeks with a water-soluble fertilizer (20-10-20, N-P-K). Plants were observed daily for the development of foliar symptoms and defoliation. Disease severity in individual plants was rated on a scale of 0 to 4 (0=no symptoms, 1=1–33%, 2=34 to 66%, 3=67–99%, and 4=plant dead) 5 weeks after inoculation (Bejarano-Alcázar et al. 1995).

Experiment II included 10 randomly selected isolates within different VCGs from olives which all had been tested in experiment I. Five of these isolates belonged to VCG1A (OVd7, OVd23, OVd34, OVd54 and OVd82), one to VCG2A (OVd151), three to VCG4B (OVd56, OVd57 and OVd60), and one was HSI (OVd55). Olive seedlings used in pathogenicity tests were propagated from semi-hardwood cuttings selected from symptomless susceptible olive trees cv. Manzanilla. The semi-hardwood olive cuttings about 0.5 to 1.0 cm diameter were cut into pieces of about 10 to 15 cm long, with the lower leaves removed, and 2 to 4 sets of leaves remaining. These cuttings were transplanted into steam-sterilized rooting media (90% perlite and 10% peat moss) under mist propagation in a greenhouse. Cuttings with healthy roots were transplanted into sterilized propagation mix (soil/sand/farmyard manure, 1:1:1) in black plastic bags (2 l). One year-old plants were inoculated by the

Table 3 Summary information for the local tester isolates of *V. dahliae* used in this study

Isolate	Geographic origin	Mutant phenotype	VCG	Host of origin
CotVd19	Manisa, Şirin	*nit*1 and NitM	1A	Cotton
OVd13	Muğla, Milas	*nit*1 and NitM	1A	Olive
OVd21	İzmir, Kemalpaşa	*nit*1 and NitM	1A	Olive
OVd22	Manisa, Kırkağaç	*nit*1 and NitM	1A	Olive
OVd34	İzmir, Tire	*nit*1 and NitM	1A	Olive
OVd35	Aydın, Yenipazar	*nit*1 and NitM	1A	Olive
OVd50	Manisa, Saruhanlı	*nit*1 and NitM	1A	Olive
OVd52	Manisa, Gölmarmara	*nit*1 and NitM	1A	Olive
OVd77	İzmir, Bayındır	*nit*1 and NitM	1A	Olive
OVd60	İzmir, Aliağa	*nit*1 and NitM	4B	Olive
OVd211	Muğla, Yatağan	*nit*1 and NitM	2A	Olive

stem-injection method (Pennisi et al. 1993). Approximately 5 cm above the soil layer, plant tissue was sterilized by swabbing 70% EtOH-soaked cotton and inoculated by injecting 500 μl of spore suspension (10^7 conidia ml^{-1}) into the stem through an incision made by a scalpel in the vascular system. The inoculated plants were then maintained in a greenhouse at 22–24°C, with a 14-h photoperiod of fluorescent light of 270 μEm^{-2} s^{-1} in a completely randomized design. The level of the disease in olive trees was evaluated in weeks 3, 6 and 9 after inoculation using the following 0 to 4 scale according to % leaves and twigs affected: 0=no sign of wilting; 1=slight marginal wilting, 1–33% of leaves with wilt; 2=34–66% of leaves with wilt; 3=pronounced defoliation, 67–99% of leaves showing wilting associated with defoliation; 4=totally defoliated dead plant (López-Escudero et al. 2004).

In both experiments I and II, mean disease severity index (DSI %) was calculated by summing the score of the 15 plants (3 replicates of 5 plants for each isolate), and expressing the value as a % using the formula:

$$DSI = [\sum(\text{rating no.} \times \text{no. of plants in rating})] \times 100] / (\text{total no. of plants} \times \text{highest disease rating})$$

. Both experiments were repeated twice and the data were presented as the mean of the two independent experiments.

Statistical analysis of the data on disease severity index was carried out using SAS software (SAS Institute Inc. 1998). Arcsine transformation was performed on data before statistical analysis. Analysis of variance was followed by mean separation using the Student–Newman–Keuls (SNK) multiple comparison tests ($P<0.05$).

Results

Generation and characterization of nitrate-non-utilizing (*nit*) mutants

One thousand, five hundred and two chlorate-resistant sectors were obtained from 207 isolates of *V. dahliae*; one isolate (OVd71) did not produce chlorate-resistant sectors. In 6 to 20 replications, each isolate produced 1 to 24 chlorate-resistant sectors. For each isolate of *V. dahliae*, 1 to 12 of the sectors was phenotyped as *nit* mutants. Some *nit* mutants had a tendency to revert back to the wild-type. The number of *nit* mutants recovered from apparently resistant sectors was 783 out of 1,502 (52.1%). Most mutants (82.9%) grew profusely on CDA with nitrite or hypoxanthine and were classified accordingly as *nit*1. About 12.8% of the *V. dahliae* grew sparsely on CDA with hypoxanthine and was classified as NitM. A low proportion (4.3%) of the *V. dahliae* mutants, which grew profusely on hypoxanthine and sparsely on nitrite, were classified as *nit*3. All *nit* mutants showed wild-type growth on PDA.

Complementation and vegetative compatibility

To assign and compare the VCGs in Turkey with those described previously, *nit* mutants generated from 207 local isolates were paired with complementary *nit* mutants of international testers and previously described VCGs from Turkey (Table 2); 90.9% of the Turkish isolates (189 isolates) produced heterokaryons with the VCG1A international *nit* mutant T9. The international VCG1A tester cot228 often produced a weak or negative reaction in pairings with complementary *nit* mutants of the same local isolates. Isolates belonging to VCG1A did not give any complementation with the other VCG testers.

Eight olive isolates (OVd151, OVd202, OVd204, OVd206, OVd208, OVd211, OVd232 and OVd234) gave a positive reaction with the Turkish testers Cy1, Ch02 and Cka1 (Dervis and Bicici 2005) along with the Israeli *nit* mutants ep8 and ep52 belonging to VCG2A. Isolates assigned to VCG2A did not produce any reactions with the testers of subgroup B of VCG2 or the other VCGs. Four olive isolates (OVd31, OVd56, OVd57 and OVd60) produced heterokaryons with the Turkish and international mutants of VCG4B (Table 2). These isolates did not give any reaction with the testers of the other VCGs including subgroup A of VCG4.

Six isolates (OVd16, OVd18, OVd38, OVd53, OVd55 and OVd221; 2.9% of the total number) could not be assigned to any known VCG. Among these isolates, OVd53 and OVd221 did not produce two *nit* mutant phenotypes and therefore could not be tested for self-compatibility. While OVd16, OVd18 and OVd38 were found to be self-compatible, OVd55 was self-incompatible. The *nit* mutants obtained from OVd16, OVd18, OVd38, OVd53 and OVd221 isolates showed negative reactions with the international tester isolates belonging to four VCGs. How-

ever, very weak indistinct reactions were observed between *nit* mutants from these five isolates and *nit* mutants from some local olive testers assigned to VCG1A. The pairings with these isolates were repeated several times with similar results. The pairing of complementary *nit* mutants of these five isolates also did not produce a heterokaryon with each other. The VCG positions of these six isolates remained uncertain. VC of isolate OVd71 was not tested because this isolate did not produce a *nit* mutant.

VCG2B, VCG4A and VCG3 were not defined among the olive isolates tested, since the isolates failed to anastomose with the VCG2B (from the isolates cot256, cot11, Ch01and Ch03), VCG4A (from the isolates 171 and 131M) and VCG3 (from the isolate 70-21) *nit* testers.

In the following experiments, *nit* mutants from olive were tested against the complementary local testers described in materials and methods (Table 3). Three distinct groups were observed which were correlated with previously characterized VCG1A, VCG2A and VCG4B. All the isolates in the first group complemented strongly with all the nine local testers (CotVd19, OVd13, OVd21, OVd22, OVd34, OVd35, OVd50, OVd52 and OVd77) of VCG1A. The isolates OVd31, OVd56 and OVd57 belonging to the second group reacted strongly with the local tester OVd60 belonging to VCG4B. Similarly, the seven isolates (OVd151, OVd202, OVd204, OVd206, OVd208, OVd232 and OVd234) belonging to the third group produced heterokaryons with the *nit* mutants from OVd211 of VCG2A.

Three types of reactions were observed at the contact of two complementary *nit* mutants. The first type was observed among all complementary *nit* mutants from the local isolates belonging to the same VCG and between the *nit* mutants from the local olive isolates and previously described Turkish cotton isolates (Dervis and Bicici 2005) as linear wild-type growth with abundant microsclerotia and dense mycelia. This type of reaction was also observed between *nit* mutants from T9 and local isolate CotVd19 from cotton. The second type of heterokaryon grew slowly with more aerial mycelium and fewer microsclerotia. This type of reaction was observed between *nit* mutants from T9 and *nit* mutants from the isolates assigned to VCG1A including the local olive testers representing VCG1A. The third type of reaction was partial compatibility, in the form of small dots of microsclerotia at the contact line. This type of reaction was seen with the isolate cot228 belonging to VCG1A.

In general, complementary heterokaryons formed between mutants of the different phenotypes (*nit*1 X NitM, *nit*3 X NitM, and NitM X NitM) in all the intra-strain pairings. The formation of heterokaryons was first seen 7 days after pairing and the observations lasted for 28 days. Based on strong complementary heterokaryosis, 201 isolates were grouped into VCGs 1A, 2A and 4B; 189 out of 208 isolates of *V. dahliae*, recovered from all the surveyed areas except Bursa, were assigned to VCG1A. Four isolates from İzmir (OVd31, OVd56, OVd57 and OVd60) were assigned to VCG4B and eight isolates from Bursa (OVd232, OVd236, OVd202, OVd204 and OVd234), Aydın (OVd208), Manisa (OVd151) and Muğla (OVd211) were assigned to VCG2A (Table 1 and Fig. 1).

Pathogenicity tests

Seventeen isolates from olive tested in experiment I were pathogenic to cotton cv. Çukurova 1518. The first symptoms developed 2 weeks after inoculation. Statistical analysis using DSI confirmed the significant differences in virulence among the isolates from the different VCGs. Collectively, isolates in VCG1A were more aggressive to cotton than those in VCG4B, VCG2A and the HSI isolate ($P<0.05$; Table 4). VCG4B and VCG2A collectively appeared to be significantly more virulent than the HSI isolate ($P<0.05$). In general, cotton plants inoculated with isolates in VCG1A showed defoliating symptoms at the squaring stage. Some plants were defoliated at early seedling stages, leaving bare stems. At maturity, these plants were totally defoliated. In contrast, inoculation with VCG4B (OVd57, OVd56 and OVd60), VCG2A (OVd151) and the HSI (OVd55) isolates led to typical symptoms of leaf chlorosis without defoliation. No symptoms developed in the control plants.

All 10 isolates from olive tested in experiment II were pathogenic to olive cv. Manzanilla. Statistical analysis using DSI confirmed the significant differences in aggressiveness among the isolates ($P<0.05$). In plants inoculated with isolates in VCG2A and VCG4B, the first symptoms developed 6 weeks after inoculation (Table 5). Chlorosis was the most common symptom observed on olive seedlings inoculated with VCG2A, VCG4B and HSI isolates. In plants

Fig. 1 Regional distribution of vegetative compatibility groups (VCGs) of *V. dahliae* from olive in western Turkey

Table 4 Disease reaction of cotton (cv. Çukurova 1518) inoculated with *V. dahliae* isolates belonging to different VCGs

Isolates	VCG	DSI (%)[a]
OVd82	1A	81.3 a
OVd34	1A	79.7 ab
OVd54	1A	78.1 abc
OVd154	1A	76.6 abcd
OVd223	1A	71.9 abcd
OVd146	1A	70.3 abcd
OVd173	1A	68.8 abcd
OVd81	1A	67.2 abcd
OVd103	1A	67.2 abcd
OVd110	1A	65.6 bcd
OVd7	1A	64.1 cd
OVd23	1A	62.5 d
OVd60	4B	39.1 e
OVd57	4B	39.1 e
OVd56	4B	37.5 e
OVd151	2A	28.1 e
OVd55	HSI	18.8 f

[a] A mean disease severity index (DSI) was calculated from each treatment by summing the score of 15 plants (3 replicates of 5 plants per isolate) using a 0–4 a scale, and expressing the value as a %. Data are presented as the mean of two independent experiments. Arcsine transformation was performed prior to statistical analysis. Means, obtained each time after inoculation, followed by different letter are significantly different according to Student–Newman–Keuls (SNK) multiple comparison tests ($P<0.05$).

inoculated with the VCG1A isolates, first symptoms developed 2 weeks after inoculation and severity increased over time. Defoliation without any chlorosis symptom was a very frequent response (Fig. 2). The VCG1A isolates induced higher incidences of disease and symptom severity than the VCG2A, VCG4B and HSI isolates, and death of plants.

Discussion

Overall, three multimember VCGs (VCG1A, VCG2A and VCG4B) were identified among the 201 isolates. VCG1A was the largest group and included 189 isolates (90.9% of all the isolates). VCG2A and VCG4B included 3.9 and 1.9% of the isolates, respectively. Different VCGs among *V. dahliae* isolates from olive trees have been reported in several Mediterranean countries previously. In Greece, two isolates from olive were assigned to VCG4, one isolate to VCG2, and four other isolates could not be grouped to any VCGs tested (Elena and Paplomatas 1998). Bao et al. (1998) tested VC among 34 *V. dahliae* isolates from different hosts including olive from different sites in Israel. According to the formation of strong complementary heterokaryons, all the Israeli isolates were grouped into two VCGs, tentatively designated as VCGI and VCGII: one isolate was assigned to VCGI and four isolates to VCGII. Cherrab et al. (2002) determined VCGs of 38 isolates of *V. dahliae* from olive trees in Morocco. Based on complementarity of *nit* mutants, 47% of the isolates were assigned to VCG4B, 32% to VCG2 (A or B), and the remaining 21% of the isolates could not be grouped to any VCGs tested. Tsror and Levin (2003) used 52 isolates of *V. dahliae* from olive trees grown at different locations in Israel to determine VCGs; 81% of the isolates were assigned to VCG4B,

Table 5 Disease reaction of olive (cv. Manzanilla) inoculated with *V. dahliae* isolates belonging to different VCGs

Isolates	VCG	Weeks after inoculation		
		3	6	9
		DSI (%)[a]		
OVd34	1A	70.3 a	96.9 a	98.4 a
OVd82	1A	50.0 b	95.3 a	98.4 a
OVd54	1A	34.4 c	70.3 b	85.9 a
OVd07	1A	26.6 cd	50.0 c	64.1 b
OVd23	1A	14.1 de	43.8 c	50.0 c
OVd55	HSI	18.8 d	25.0 d	26.6 d
OVd60	4B	0.00 e	1.6 e	17.2 d
OVd57	4B	0.00 e	9.4 e	15.6 d
OVd56	4B	0.00 e	4.7 e	12.5 d
OVd151	2A	0.00 e	3.0 e	13.2 d

[a] A mean disease severity index (DSI) was calculated from each treatment by summing the score of 15 plants (3 replicates of 5 plants per isolate) using a 0–4 a scale, and expressing the value as a %. Data are presented as the mean of two independent experiments. Arcsine transformation was performed prior to statistical analysis. Means, obtained each time after inoculation, followed by different letter are significantly different according to Student–Newman–Keuls (SNK) multiple comparison tests

the remaining isolates to VCG2A. Bellahcene et al. (2005) obtained 25 *V. dahliae* isolates from olive trees in Algeria, with all the olive isolates grouped into a single VCG. Collado-Romero et al. (2006) used 45 olive isolates from Spain, Italy, Syria, Cyprus and Greece. According to their results, 9 isolates from Spain were assigned to VCG1A; 26 from Spain, Italy, Syria and Cyprus to VCG2A; 9 isolates from Spain and Greece to VCG4B; and 1 isolate from Cyprus was HSI.

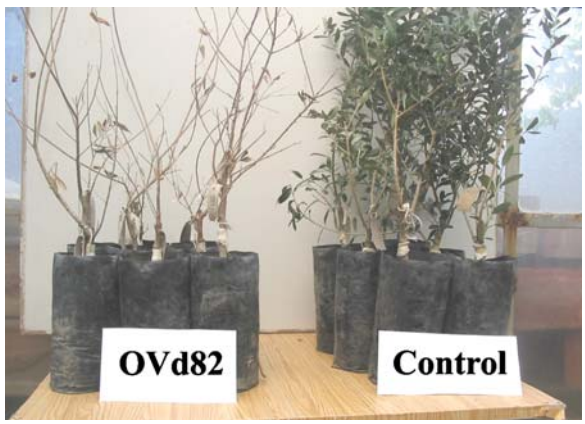

Fig. 2 Disease reactions in olive plants of cv. Manzanilla, 7 weeks after stem inoculation with isolate OVd82 of *V. dahliae* VCG1A. Inoculated plants showing typical defoliating symptoms are on the left; non-inoculated control plants are on the right

Our study showed that the isolates in VCG1A were distributed across the region except in Bursa and were the most prevalent isolates in the western part of Turkey. All isolates within a VCG complemented one another very strongly and no cross-reaction occurred among the isolates of the different VCGs. These results may indicate the presence of homogeneity within the each VCG.

Reactions between all the local VCG1A isolates and tester T9 were positive but occurred three to 5 days later compared to the remaining isolate pairings. This delay may be attributed to the fact that T9 is a foreign isolate from the USA and from a host plant different from olive. However, abundant and more robust prototrophic growth was observed between CotVd19 and T9 (both from cotton plants). Supporting our conclusions, reactions between the *nit* mutants from the isolate CotVd19 and the *nit* mutants from 189 isolates assigned to VCG1A including local olive testers were very strong and quick. Furthermore, eight local olive testers belonging to VCG1A produced very strong and quick heterokaryons with all the isolates assigned to VCG1A. Since T9 belongs to subgroup VCG1A (Bell 1994), all isolates strongly compatible with it regardless of the speed of heterokaryon formation should accordingly be assigned to VCG1A (Korolev et al. 2001a). Nevertheless, complementation tests of VCG1B are required for the final confirmation as suggested by Korolev et al. (2001a). Although VCG1A has been reported from different host plants (Bell 1994; Chen 1994; Collado-Romero et al. 2006; Daayf et al. 1995; Jiménez-Díaz et al. 2006; Korolev et al. 2001a; Zhengjun et al. 1998), it has not been recorded to our knowledge for *V. dahliae* from olive with the exception of the work in Spain by Collado-Romero et al. (2006). On the other hand, there are other reports on the presence of the D pathotype of *V. dahliae* from olive in Spain (López-Escudero and Blanco-López 2001; Mercado-Blanco et al. 2003).

Olive isolates tested in greenhouse experiments I and II were pathogenic to cotton cv. Çukurova 1518 and olive cv. Manzanilla. In plants inoculated with VCG1A isolates, disease symptoms developed earlier, were more severe, and plants died earlier than those with VCG4B and VCG2A isolates. VCG1A of *V.*

dahliae in Turkey was clearly distinct in its virulence characteristics showing all symptoms of the defoliating type (D pathotype). Since VCG1A represents the D pathotype of *V. dahliae*, its prevalence is especially crucial for the countries of the Mediterranean basin.

In conclusion, olive trees from diverse locations in western Turkey are infected with three major VCGs (VCG1A, 2A and 4B) of *V. dahliae* and thus may serve as primary sources of the pathogen. Data on the VCG distribution of *V. dahliae* and their relative aggressiveness would enable a more accurate evaluation of potential damage and control measures and also would be of practical importance especially in new plantation areas. These findings are potentially important to the olive industry in Turkey and would enable a more accurate selection of isolates when screening resistant and tolerant olive lines in breeding programmes.

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